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Maple Sirup

XIII. Sterilizing Effect of Sunlight on Maple Sap in Transparent Tubes[1]

Within the last few years, transparent plastic tubing has been employed for the collection and transportation of maple sap directly from the taphole of the trees to the evaporator or roadside tank. Thus, in any one sugar bush, several miles of interconnected tubing are often required. The sap contained in this tubing can serve as a medium for growth of adventitious microorganisms. The deleterious effect of these microorganisms on the quality of maple sirup has been pointed out in other publications (Edson *et al.*, 1912; Fabian and Buskirk, 1935; Hayward and Pederson, 1946; Owens, 1949; Holgate, 1950; Porter *et al.*, 1954; Naghski and Willits, 1955; Naghski *et al.*, 1957). A recent study in this laboratory (Naghski and Willits, 1953) demonstrated that the growth of microorganisms in contaminated maple sap was reduced by sunlight transmitted through transparent plastic collection bags.

This phenomenon of induced changes in microorganisms caused by exposure to sunlight is not new. The first to observe the germicidal effect of sunlight were Downes and Blunt (1877, 1879). Since then papers have appeared which reported on the effect of sunlight on sporulation (Arloing, 1885a, b), germination (Roux, 1887; Ward, 1893a, b), and on growth rates (Arloing, 1885a, b; Ward, 1893a, b).

Other investigations (Coblentz and Fulton, 1924; Meader, 1926; Buchbinder *et al.*, 1941; Buttolph, 1955) have demonstrated that the most lethal portion of the sunlight spectrum is in the region of 265 mμ. Whether the germicidal action of sunlight or ultraviolet radiation is the result of direct action on some cell component and/or indirectly through the formation of some substance in the suspending medium as a result of irradiation has not been resolved (Jagger, 1958).

Polyvinyl and polyethylene plastic tubings being used to transport maple sap are partially transparent in the visible and in the ultraviolet region of the spectrum. This study was designed to show whether or not sufficient sunlight radiation in the ultraviolet region is transmitted through this plastic tubing to effectively control or reduce the microbial flora of the contained sap.

[1] Mention of trade names does not imply endorsement by the U. S. Department of Agriculture over similar products not mentioned.

[2] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Materials and Methods

Organisms. Four bacterial strains, isolated previously from naturally contaminated maple sap, were employed. The following temporary designations have been assigned (Naghski *et al.*, 1957): *Pseudomonas*-11 (deposited in the Northern Regional Research Laboratory and labeled NRRL B-1888), *Pseudomonas*-25 (NRRL B-1890), *Flavobacterium*-387 (NRRL B-1887), and *Flavobacterium*-583 (NRRL B-1889). Stock cultures were maintained on nutrient agar slants. Inocula were grown on tryptone glucose yeast extract (TGY) agar for 24 hr at 27 C, washed from the surface with sterile distilled water, and diluted to the desired concentration. Suspensions of mixed inocula were prepared by combining approximately equal numbers of the four strains. Initial concentrations of organisms in inoculated sap were estimated by plating with TGY agar and incubation at 27 C for 3 days. Sterile, frozen maple sap was thawed, as described previously (Naghski *et al.*, 1957), transferred aseptically to sterile containers, and inoculated with the mixed suspension.

Procedure for irradiation by sunlight. Tubing of four different materials (Pyrex, Vycor, polyethylene, and polyvinyl) were used. Polyvinyl tubing was obtained from two sources. A difference in composition may be inferred as polyvinyl no. 2 was more transparent to light in the 300 to 375 mμ region than polyvinyl no. 1. The diameter and wall thickness of the tubes were comparable to and typical of tubing used in sap collection. The dimensions of each of the tubes are given in table 1.

The Pyrex and Vycor tubes were sterilized by autoclaving. The plastic tubes were sanitized by washing with a detergent solution followed by rinses with hot tap water and, finally, with sterile distilled water. All tubes were closed with sterile stoppers after the inoculated sap had been introduced. The tubes of the five types were divided into three groups (designated A, B, and C) and treated as follows:

Group A. Five ml of inoculated sap were placed in seven tubes of each of the five tube types. From these,

five sets were assembled, each containing seven tubes of the five tube types. These five sets were placed out-of-doors and located with an unrestricted exposure to the sky. Another five sets, prepared in an identical manner, were also placed outdoors in the same location but in a light-proof box. Periodically, one set of tubes from each of the two subgroups (exposed and non-exposed) was removed and the bacterial population estimated by the plate count method.

TABLE 1

Dimensions of different types of tubing employed for exposure of inoculated maple sap to irradiation by sunlight

	Type of Tubing				
	Polyvinyl		Polyethylene‡	Pyrex§	Vycor§
	No. 1*	No. 2†			
	Dimensions (mm):				
Outer diameter	7	10	10	15	15
Inner diameter	5	8	7	13	13
Wall thickness	1	1	1.5	1	1
Length	270	150	180	125	125

* Mapleflo, obtained from the Minnesota Mining and Manufacturing Co., Irvington, New Jersey.

† Purchased from A.C. Lamb and Sons, Liverpool, New York.

‡ Obtained from Skyline Industries Sales, Inc., Titusville, Pennsylvania.

§ Obtained from Corning Glass Company, Corning, New York.

Group B. Similar to group A except that the five tube types contained only sterile sap. This group of tubes was also divided into two subgroups, exposed and nonexposed, as described for group A. Sets of tubes were removed at the same time as those of group A and plated for the presence of contamination. This group of tubes (B) served as a check on the sterility of the uninoculated sap and of the tubes employed in this study.

Group C. This group was prepared and treated in the same manner as group B except that each set consisting of only two tubes of each of the five types was designed to investigate the germicidal properties of sunlight-irradiated maple sap. The two subgroups (exposed and nonexposed) were left out-of-doors for the duration of the experiment. The sterile sap was then removed aseptically, transferred to a sterile test tube, inoculated with a small volume (about 0.1 ml) of a mixed bacterial suspension, and incubated at 27 C. Growth in these inoculated saps was followed for 2 weeks by periodically measuring their turbidities at 540 mμ with a Coleman[3] model 14 spectrophotometer.

The spectral transmission of the plastic tube materials was determined on a cut, single thickness section of the tubing mounted in a sandwich between quartz plates moistened with glycerin to reduce surface scattering.

The temperatures and sunshine data were obtained

[3] Coleman Instruments, Inc., Maywood, Illinois.

TABLE 2

Effect of sunlight on a mixed inoculum in maple sap contained in various types of tubing

Date: March, 1958	Exposure	Amount Sunshine per Day	Avg Temp	Bacterial Counts per Ml									
				Polyvinyl no. 1		Polyvinyl no. 2		Polyethylene		Vycor		Pyrex	
				Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered
	days	*min*	*C*										
5	0	—	—	26,000	26,000	26,000	26,000	26,000	26,000	26,000	26,000	26,000	26.000
5	0½	191	5.0										
6	1½	103	4.4	112	15,300	15	10,800	22	12,200	25	17,100	12	22,800
7	2½	673	5.6	5	43,100	0	33,700	0	32,600	0	27,600	5	60,700
8	3½	679	3.9	1	442,000	0	213,000	0	370,000	0	511,000	0	257,000
9	4½	640	1.1	0	468,000	0	2,980,000	0	4,420,000	0	3,830,000	0	753,000
10	5½	614	6.7										
11	6½	600	6.1										
12	7½	510	4.4	0	37,200,000	0	41,700,000	0	44,000,000	0	41,000,000	0	28,700,000
13	8½	443	2.2										
14	9½	40	2.2	0	68,800,000	0	89,400,000	0	73,000,000	0	59,000,000	0	27,500,000
15	10½	482	4.4										
16	11½	99	3.3										
17	12½	377	3.3										
18	13½	447	4.4										
19	14½	0	2.2										
20	15½	0	1.1	0	55,000,000	0	92,000,000	0	78,000,000	0	65,000,000	0	57,000,000

from the monthly climatological report of the United States Weather Bureau for the area including the laboratory during the time of the experiments.

Results

Disinfection by sunlight. The germicidal effect of sunlight on bacteria inoculated in sap is shown in table 2. The data show a comparison of the numbers of surviving microorganisms in sap contained in the different types of tubes exposed to sunlight with those in identical tubes but not exposed. Destruction of the inoculum in all exposed tubes was rapid, being essentially complete in 1 day, with less than 2 days being required to diminish the counts from 26,000 to 5 per ml. Only 3 days were needed for complete sterility in all tube types. The weather during this early period of exposure was cold (approximately 5 C), with a large amount of sunshine which proved ideal for showing the pronounced effects of sunlight irradiation. Growth of the inoculum in sap in the nonexposed tubes showed a progressive increase after the usual lag phase.

Since most of the destruction of the inoculum by sunlight occurred during the first 1½ days of exposure, and, since it was suspected that the greatest reduction occurred during the first half day, a second experiment was conducted in which samples were taken at shorter time intervals to obtain a more accurate measure of the rate of destruction. The results of the second experiment are shown in table 3. The growth rates obtained in nonexposed samples were somewhat higher than those obtained in the previous experiment (table 2) and can be attributed to the 3 to 5 C higher temperatures. Also, the higher destruction rates during the early exposure period are the result of the increased amount of sunshine that occurred when the second experiment was run. By comparing the numbers of surviving microorganisms after 3 hr of exposure, the relative amounts of lethal radiation transmitted by the different types of tubing can be estimated. It can be seen (table 3) that the largest amount of lethal radiation was transmitted by the Vycor tubes, followed by Pyrex, polyethylene, and the polyvinyls in decreasing order.

Figure 1 shows the transmittance curves for the different types of tubes and also a curve representing the relative bactericidal effectiveness of radiation in the ultraviolet portion of the spectrum (Buttolph, 1955). Since per cent transmittance by the different tube types is not the same over the spectral region of optimal germicidal activity (235 to 310 mμ), no clear-cut comparisons can be made. However, it can be seen that the per cent transmittancy of Vycor in the region of maximum germicidal effectiveness is the greatest of the materials tested and is followed in order by Pyrex and polyethylene, with the polyvinyls transmitting only above 290 mμ. A comparison of the rate of reduction in the microbial population of inoculated sap in the five tube types corresponds favorably with the per cent transmission of ultraviolet radiation by these tubes in the spectral region of maximum germicidal activity.

Growth in irradiated sap. The inhibitory effect of sunlight-irradiated sap on microbial growth is shown in figure 2. Sterile sap was exposed, as described above, to sunlight for 3 days (April 1 to 4, 1958) and then seeded with a mixed bacterial suspension (initial concentration of about 2.5×10^5 cells per ml). Growth of the inoculum in nonexposed sap samples stored under the same conditions was virtually the same for all five types of tubing. A composite curve was therefore constructed from these five essentially identical growth curves and is shown in figure 2. The most pronounced effect of sunlight radiation is seen in sap which was exposed in the Vycor tubes, that is, where complete inhibition of growth of the inoculum occurred. Sap exposed in the other tube types exhibited varying degrees of prolongation of the lag phase, but they did not cause complete inhibition of growth. A slight lowering of maximal growth level occurred in all of the sap samples regardless of the tube type in which they were exposed to sunlight irradiation.

TABLE 3

Effect of sunlight on a mixed inoculum in maple sap contained in various types of tubing

Date: April, 1958	Exposure	Amount Sunshine per Day	Avg Temp	Bacterial Counts per Ml									
				Polyvinyl no. 1		Polyvinyl no. 2		Polyethylene		Vycor		Pyrex	
				Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered	Exposed	Covered
	hr	*min*	*C*										
1	0	—	—	30,000	30,000	30,000	30,000	30,000	30,000	30,000	30,000	30,000	30,000
1	3	—	—	4,600	21,400	660	13,400	257	19,900	21	29,600	143	23,300
1	6	530	8.3	38	24,000	6	20,200	0	22,500	0	23,200	3	24,000
2	24	744	8.9	8	30,000	0	23,000	0	33,000	0	58,000	0	44,000
3	48	670	8.3	2	840,000	0	770,000	0	1,050,000	0	700,000	0	1,310,000
4	72	590	8.3	0	18,700,000	0	20,700,000	0	11,800,000	0	24,200,000	0	30,800,000

Discussion

These experiments were conducted during the maple sap season so that the sunlight spectrum and the outdoor temperatures would be typical of those encountered in maple sap operations.

These results confirm the germicidal effectiveness of sunlight against microorganisms in maple sap (Naghski and Willits, 1953) and show that the degree of microbial destruction is related to the dosage of ultraviolet radiation to which they are exposed. The germicidal effects obtained when exposed saps were contained in Vycor and in Pyrex could not be attributed to soluble toxic substances derived from the walls of the tubing since Vycor and Pyrex are insoluble under the conditions of these experiments.[4] Nor could the germicidal effects obtained when the saps were contained in plastic tubes be attributed to soluble toxic substances derived either from the plasticizer or decomposition products of the plastic since growth of the inoculum in nonexposed sap occurred at the same rate for all tube types. This confirms the work of Walter *et al.* (1958) who reported no observable effect of plastics on growth of microorganisms.

[4] Data supplied by Corning Glass Company, Corning, New York.

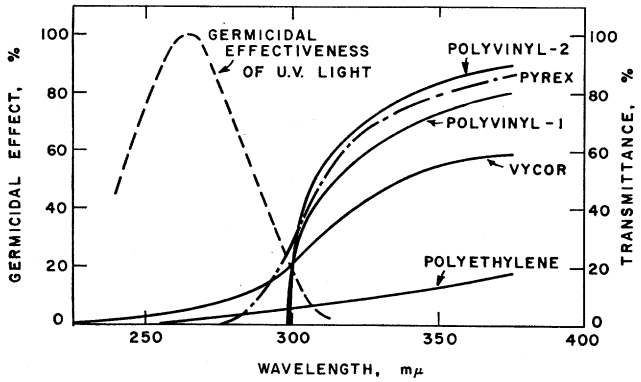

Figure 1. Spectral transmittance of several types of tubing and a curve representing relative germicidal effectiveness of radiant energy (after Buttolph, 1955).

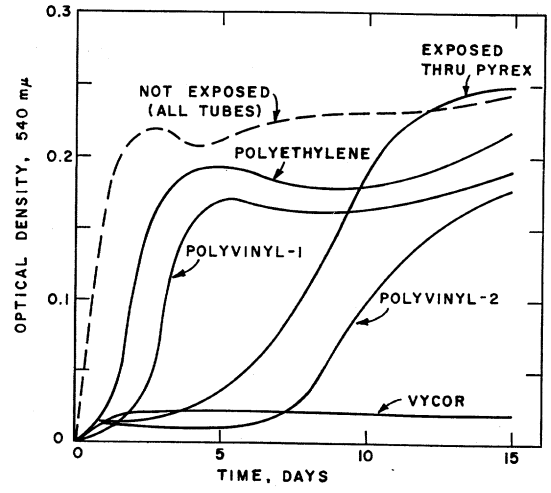

Figure 2. Growth of a mixed bacterial inoculum suspended in sap which had been exposed to sunlight radiation for 3 days while being contained in various types of transparent tubing.

When maple sap is transported through plastic tubing (transparent to ultraviolet radiation, especially in the region below 310 mμ), two phenomena occur. The transmitted ultraviolet radiation has a germicidal effect and the temperature influences the microbial growth rate. During that part of the maple season when temperatures are near freezing (usually early in the season) the growth rate is slow and the effect of ultraviolet radiation predominates and the transported sap will have a low microbial count. Under the same low temperature conditions, the ultraviolet radiation effect is lessened when days are cloudy or the tubing is shadowed or covered by snow. The net effect of these conditions is that the cell count remains low because of the low temperature, even though there is not enough sunlight to cause sterilization of the sap.

When warmer weather prevails, usually toward the end of the sap season, the growth rate is greatly accelerated and, in spite of the ultraviolet irradiation, an increase in cell count occurs. This becomes pronounced with diminished sunlight caused by cloudy weather and by warm nights. The increased flora of the sap, if excessive, will result in the production of a lower grade (that is, darker) maple sirup as well as a buildup of microbial debris on the inner surface of the tubing. The latter, if not corrected by sanitizing and washing could, on continued use of the tubing, cause spoilage of the sap crop of the following year and, because of its dark color, cause diminished transparency of the tubing.

This work has shown that sap irradiated with sunlight acquires germicidal properties. This is consistent with the observations of Coblentz and Fulton (1924), Blank and Arnold (1935), Baumgartner (1936), Stone *et al.* (1947), Novick and Szilard (1949), and Northrop (1957).

These two effects of sunlight irradiation on sap being transported by transparent, plastic tubing, the direct germicidal action on the microorganisms and the induced germicidal properties of sap, contribute to the production of high quality maple sirup.

Acknowledgments

We wish to thank Miss Anne M. Smith for making the transmittance measurements on the tubing and to Mr. R. A. Bell for technical assistance.

Summary

When inoculated maple sap, contained in different types of tubes, is exposed to sunlight, the relative amount of germicidal activity is related to the transparency of the tubing to ultraviolet radiation.

The germicidal activity of sunlight is due mainly to ts effect on the microbial cells and, to a lesser extent, n the sap. The net effect, microbial growth, is influ-nced by the environmental temperature as well as by ıltraviolet irradiation.

The cell count in sap in plastic tubing used for the ransportation of sap in sugar bushes will be influenced oy the transparency of the tubing to ultraviolet irradia-ion, to the length of the day, the amount of sunlight, ınd to the temperature of the sap.

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